

On-line Nano LC/Nanospray MS for the Analysis of Protein Digests

INTRODUCTION

The characterization of proteins and peptides originating from complex biological samples often require sample isolation, cleanup and separation using HPLC and 2-D gel electrophoresis (see also Application Note 514) prior to MS or MS/MS analysis. The amount of material available from such samples is usually limited and sample concentrations are very low. Therefore, low limits of detection are necessary for the

analysis of these samples which can be obtained by using Nano HPLC–Nano ESI-MS-MS.

The sensitivity of Nano HPLC compared to conventional HPLC increases approximately 3700 times (see also Application Note 513). To benefit from this theoretical increase, hyphenation to MS is a necessity where the entire column effluent is sprayed directly onto the sampling cone of the ESI interface.

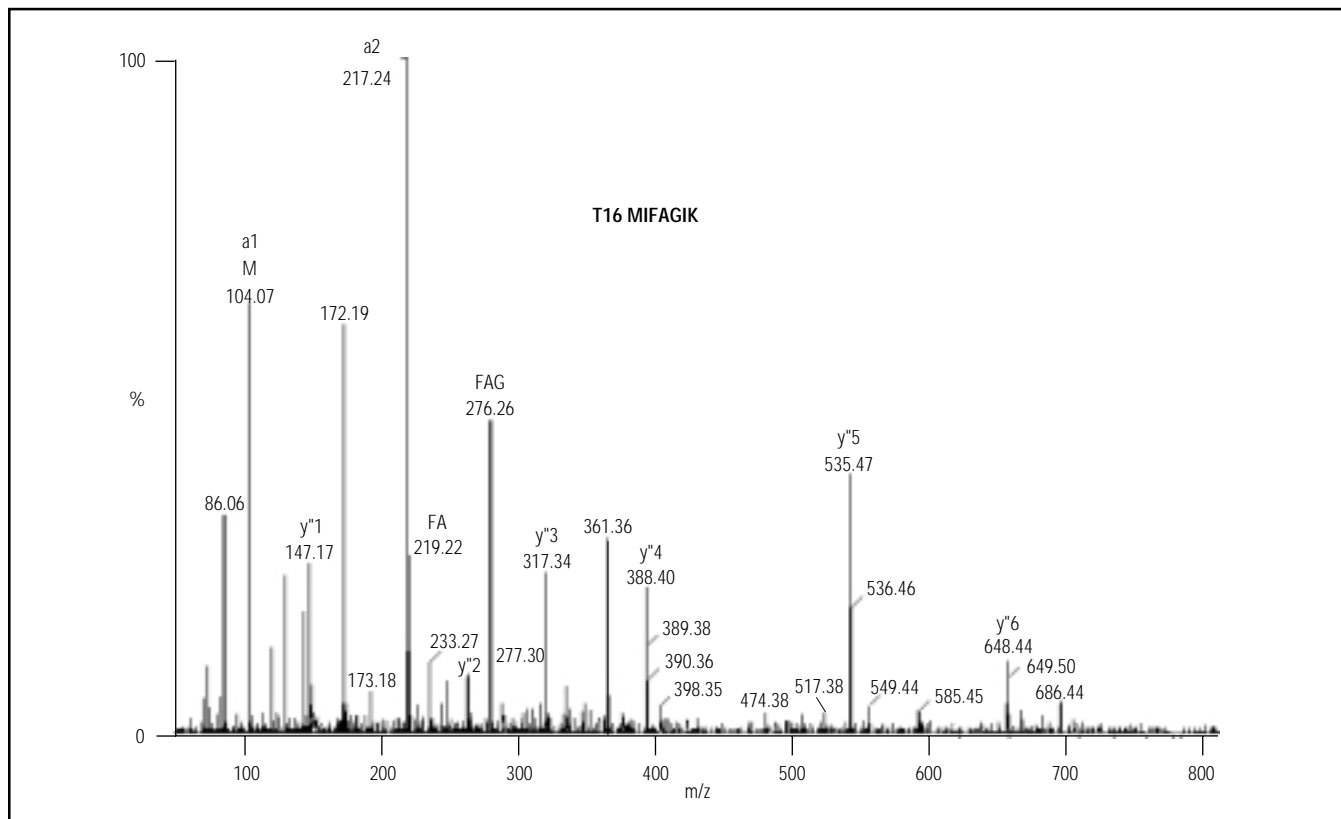


Figure 1. MS/MS spectrum of a cytochrome C fragment after tryptic digestion and separation on a 75 μ m I.D. x 15 cm nanocolumn, packed with C18, at the 800 amole level.

RESULTS AND DISCUSSION

To demonstrate the limits of detection that can be achieved with such a system, a bovine cytochrome C digest was analyzed. Automated 100 nL injections were conducted using a FAMOS™ micro autosampler. On a 75 µm I.D. x 15 cm, C18 nanocolumn detection limits of 800 amole in the MS/MS mode were obtained. A typical example is shown in Figure 1.

A synthetic peptide mixture—typical of the structures found in MHC antigen analysis—was also studied. The Figure 2 (A) shows the base peak intensity chro-

matogram of the MHC class I peptide mixture at 170 fmole separated on a 75 µm I.D. x 15 cm, C18 nanocolumn. The flow rate was 150 nL/min. Figure 2 (B) shows the MS spectrum of a peptide eluting at 18.43 min (injected amount 1.7 fmole).

REFERENCES

1. Dr. P. van Veelen, Leiden University Hospital, The Netherlands.
2. Dr. J. I. Langridge and Dr. R. S. Bordoli, Micromass, U.K.

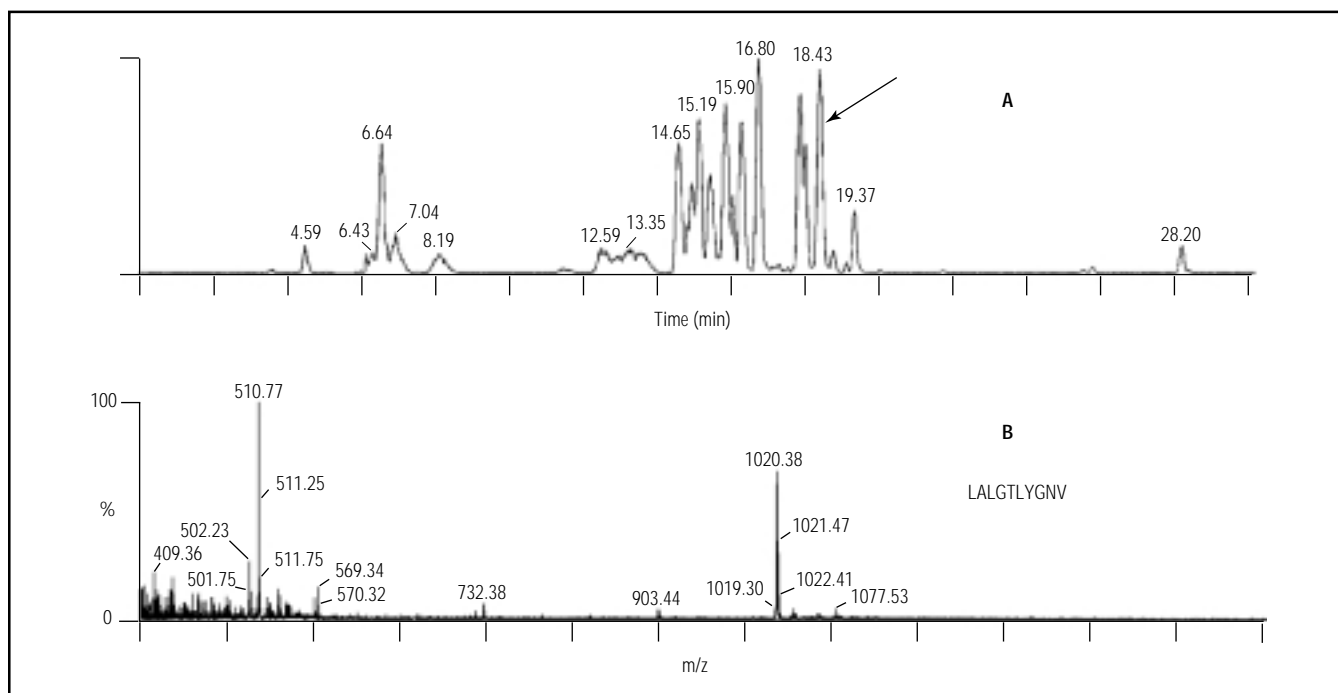


Figure 2. A) Base peak intensity chromatogram of MHC class I peptides. B) Nano-ESI-MS spectrum of the peak eluting at 18.43 min, 1.7 fmol.

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